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Comparison of simultaneous ^{99m}Tc-HMPAO and ^{111}In oxine labelled white cell scans in the assessment of inflammatory bowel disease

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Abstract. Forty-seven patients, 29 with chronic inflammatory bowel disease (IBD) and 18 with presumed irritable bowel syndrome, including one with uncomplicated diverticular disease, were studied with simultaneous technetium-99m hexamethylpropylene amine oxime and indium-111 oxine labelled leucocyte scans performed at 1, 3 and 24 h. Twenty-seven patients with IBD had active disease as judged by clinical and laboratory criteria and all of these had positive scans with both agents. No false positive studies were obtained. The 1-h ^{99m}Tc-HMPAO WBC scans showed the same distribution to disease as the 3-h ^{111}-In WBC scans, with no difference in intensity ($P<0.92$); they showed more extensive disease ($P<0.02$) and more intense uptake ($P<0.001$) than did the 1-h ^{111}-In scans. The 3-h ^{99m}Tc-HMPAO WBC scans showed more extensive disease ($P<0.002$), with greater intensity ($P<0.0005$), than did the 3-h ^{111}In WBC scans. Physiological bowel activity on 3-h ^{99m}Tc-HMPAO WBC scans was present in 12 patients but was faint and did not interfere with assessment of disease extent and activity. It is concluded that in terms of isotope availability, radiation dosimetry and image quality, ^{99m}Tc-HMPAO is the agent of choice in detecting active IBD, with localization of disease possible at 1-h after re-injection and optimal resolution and definition of disease extent at 3 h. A negative scan reliably excludes active disease.

Key words: Inflammatory bowel disease – White cell scans

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Introduction

The diagnosis of chronic inflammatory bowel disease (IBD) is usually based on clinical, radiographic and histological findings. A recent study suggests that the annual incidence of Crohn's disease has increased (from 1/100000 to 6.67/100000) over the last 25 years [1]. Therefore the number of patients referred for investigation has also grown considerably.

Standard radiographic and endoscopic examinations are time-consuming, require patient preparation and may be uncomfortable. In patients with Crohn's disease both small and large bowel barium studies may be required to visualise the extent of the disease, and in acutely ill patients the scope for conventional investigation may be limited. Labelled white cell studies have the advantage that they require minimal patient preparation, can visualise most of the small and large bowel, may reflect activity of disease and can be performed in acutely ill patients.

Imaging using indium-111 labelled leucocytes has been shown to correlate closely with radiology [2], endoscopy and histology [3] for disease extent and activity in patients with IBD. Imaging with technetium-99m hexamethylpropylene amine oxime labelled granulocytes has the advantages of greater isotope availability, improved image resolution and better radiation dosimetry. It is a highly sensitive and specific method of detecting inflammation [4] and in patients with Crohn's disease has been shown to correlate well with small bowel radiology [5], barium enema, endoscopy and surgery [6]. Correlation with clinical disease activity scoring is less good but improves when taken in conjunction with laboratory parameters of inflammation [6]. Potential disadvantages of image interpretation are renal and biliary excretion, which may obscure inflammation in adjacent areas of bowel, and "physiological" 24-h gut activity, which may produce appearances indistinguishable from those of abscesses [7].

Because of the potential advantages of imaging with ^{99m}Tc-HMPAO labelled granulocytes, this study was designed to compare simultaneous ^{111}In oxine and ^{99m}Tc-HMPAO labelled granulocyte scans in patients with proven Crohn's disease and ulcerative colitis and in pa-

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tients with suspected irritable bowel syndrome (IBS) as negative controls, in order to decide whether ^{99m}Tc-HMPAO labelling could replace ^{111}In in this group of patients.

Materials and methods

Patients. Forty-seven patients with IBD or probable IBS (22 males, 24 females, age range 17–70, mean 33.3 years) were studied; some were referred from ward, others were outpatients. Twenty patients had Crohn's disease, nine had ulcerative colitis, 17 had IBS and one had diverticular disease.

The diagnosis was made on the basis of standard clinical, radiological and histopathological criteria. Disease activity was assessed using a combination of clinical disease activity scoring [8] and laboratory parameters such as C-reactive protein and erythrocyte sedimentation rate. Written informed consent was obtained and the study was approved by the Hospital Ethics Committee and the Administration of Radioactive Substances Advisory Committee (ARSAC).

Procedure

Patients attended the Nuclear Medicine Department on two consecutive days. On the first day, cell labelling, re-injection and 1- and 3-h images were performed (time in department approximately 6 h) and on the second day 24-h images were taken (time approximately 45 min).

White cell labelling

The patient was venesected of 90 ml of whole blood via a 19 G butterfly cannula over 1–2 min.

^{111}In labelling. Sixty millilitres of the whole blood was drawn into a heparinised syringe. Fifty millilitres was allowed to stand with 2 ml of 2% methyl cellulose solution for 45 min. The remaining 10 ml was transferred to a 15-ml conical screw-capped tube and centrifuged at 1000 *g* for 5 min to provide platelet-poor plasma (PPP).

Twenty millilitres of supernatant from the sedimented blood sample was removed and centrifuged at 80–90 *g* for 7 min to produce a WBC pellet. The supernatant was removed and the WBC resuspended in 15 ml 0.9% saline. Approximately 20 MBq ^{111}In oxine (Amersham International plc) was added to the cell suspension, followed by incubation for 15 min. After this time the labelled cells were mixed with half the volume of the PPP and centrifuged at 80–90 *g* for 7 min. The supernatant containing non-cell-bound ^{111}In was removed and the radioactivity counted. The ^{111}In labelled cells were resuspended in 10 ml 0.9% saline and the activity counted. The binding efficiency wa determined. The radiolabelled cells were finally drawn into a fresh 20-ml syringe together with the remainder of the PPP.

^{99m}Tc-HMPAO labelling. The technique for labelling cells with ^{99m}Tc-HMPAO is described [9] as the Mark 1 labelling protocol. This was the first ^{99m}Tc-white cell technique adopted at our institution and, as we wanted to compare our routine techniques used for ^{111}In labelling and ^{99m}Tc-HMPAO labelling, we continued with this technique.

Thirty millilitres of blood was drawn into a 60-ml syringe containing 3 ml of anticoagulant citrate dextrose (ACD) solution. After mixing, the blood was transferred to a 30-ml universal container and 2 ml Hespan (Dupont) was added and mixed. The blood was allowed to stand for approximately 45 min until the red cells settled. The leucocyte-rich plasma (LRP) was aspirated and centrifuged at 150 *g* for 5 min. The platelet-rich plasma (PRP) was removed and kept. A kit of ^{99m}Tc-HMPAO (Ceretec, Amersham International plc) was prepared using fresh eluate from a generator (the eluate must be less than 2 h old and from a generator which has been eluted within the last 24 h). Then, 1 GBq ^{99m}Tc in 2 ml was added to the kit and the contents quickly transferred to the white cell pellet and gently mixed and incubated for 10 min: although adding the ^{99m}Tc-HMPAO to the cells immediately after preparation is not a stated requirement in reference 9, it is a requirement of the protocol (C. Lazarus and K.K. Solankji, personal communication). Meanwhile the PRP was centrifuged at 2000 *g* for 5 min to produce cell free plasma (CFP). After incubation 3 ml CFP was added to the white cells and centrifuged for 5 min at 150 *g*. The supernatant was removed and the radioactivity measured. The labelled white cell pellet was resuspended in a further 3 ml CFP and the radioactivity measured. The binding efficiency of the leucocyte labelling was determined using the method described in technique Mark 1 [9]. The radiochemical purity of ^{99m}Tc-HMPAO and cell viability were not routinely tested. The volumes of both preparations to give the required dose were calculated (^{111}In: 5–10 MBq; ^{99m}Tc: 100–150 MBq). Immediately before injection these volumes were drawn into the same syringe and mixed. The labelled white cells were slowly reinjected using a 19 G needle.

Imaging protocol

Equipment. Images were obtained using a Scintronics LFOV gamma camera and medium energy parallel-holed collimator.

Energy settings. A 140 keV 20% window was used for the ^{99m}Tc-HMPAO images and 173 keV, 247 keV 20% windows were used for the ^{111}In images.

Acquisition parameters. The camera was peaked to the selected isotope. Images were acquired for 5 min into a 128 × 128 word matrix.

Images acquired. Anterior and posterior abdominal views were obtained at 1, 3 and 24 h. A posterior chest image was also obtained at 1 h to check that there was no retention of labelled white cells. Digital images were interpolated and enhanced for optimal data presentation and recorded onto single sided emulsion film.

Reporting

The images were reported by two experienced observers (IF and SC) who were blind to the diagnosis and laboratory findings. The scans obtained with each isotope were reported on separate occasions in a different random order and without knowledge of the findings obtained with the other isotope.

A predefined bowel map which divided the bowel into seven segments (small bowel, caecum, ascending colon, transverse colon, descending colon, sigmoid and rectum) was used to assess each of six scans (1-, 3- and 24-h ^{111}In and ^{99m}Tc-HMPAO) obtained per patient. The activity of each segment was graded for both

intensity and distribution of uptake according to a predefined scale of 0–3 (0 = no uptake; 1 = faint uptake < bone marrow; 2 = uptake < liver > bone marrow; 3 = uptake > liver).

Statistics

A series of comparisons were made between the 1-h ^{99m}Tc-HMPAO and the 1-h ^{111}In WBC scans, the 3-h ^{99m}Tc-HMPAO and the 3-h ^{111}In WBC scans and the 1-h ^{99m}Tc-HMPAO and 3-h ^{111}In WBC scans. The difference in extent of diseased bowel shown by each agent was analysed using the Wilcoxon matched pairs signed rank test and a standard statistics computer software package (MINITAB Inc., 3081 Enterprise Drive, State College, PA 16801-2756, USA; British distributors: Clecom Ltd, Research Park, Vincent Drive, Edgbaston, Birmingham, B15 2SQ). The difference in resolution was analysed using a weighted intensity score derived from the intensity of uptake score and segmental bowel map; each scan (seven segments) would have a maximum score of $3 \times 7 = 21$. The difference between the ^{99m}Tc-HMPAO and the ^{111}In WBC scans versus the average difference between the two was plotted at 1, 3 and 24 h to investigate whether one agent consistently produced higher scores. The Wilcoxon matched pairs signed rank test (MINITAB Inc) was used to compare the two agents at each time interval.

Results

Labelling

The mean radiochemical purity of the ^{99m}Tc-HMPAO white cells was 37.2% (range 16.2%–66.6%). The mean radiochemical purity of the ^{111}In white cells was 61.9% (range 39.0–82.7%). When injected, at least 91% of the ^{99m}Tc and 97% of the ^{111}In were associated with white cells.

Reporting

No scans showed significant lung uptake.

Of 47 patients investigated, 27 (nine with ulcerative colitis and 18 with active Crohn's disease) were thought from scan findings to have active disease, whilst 20 patients (two patients with inactive Crohn's disease and 18 with IBS/diverticulosis) had negative scans. Both observers reported positive scans with both agents in the active group and negative scans in the inactive group: there were no false positives or false negatives with either agent when assessed against clinical and laboratory criteria of activity (Table 1).

All of the ^{99m}Tc-HMPAO WBC scans that were positive for active disease at 3 h showed activity at 1 h, compared with 24 of 27 (89%) ^{111}In WBC scans. Only one patient with ulcerative colitis had a negative 1-h ^{111}In

Table 1. Scan diagnosis vs disease activity

	^{99m}Tc-HMPAO no. scans positive			^{111}In no. scans positive		
	1 h	3 h	24 h	1 h	3 h	24 h
Disease positive:						
U/C ($n=9$)	9	9	9	8	9	6
Active Crohn's disease ($n=18$)	18	18	15	16	18	10
Disease negative:						
Inactive Crohn's disease ($n=2$)	0	0	1	0	0	0
IBS ($n=18$)	0	0	13	0	0	0

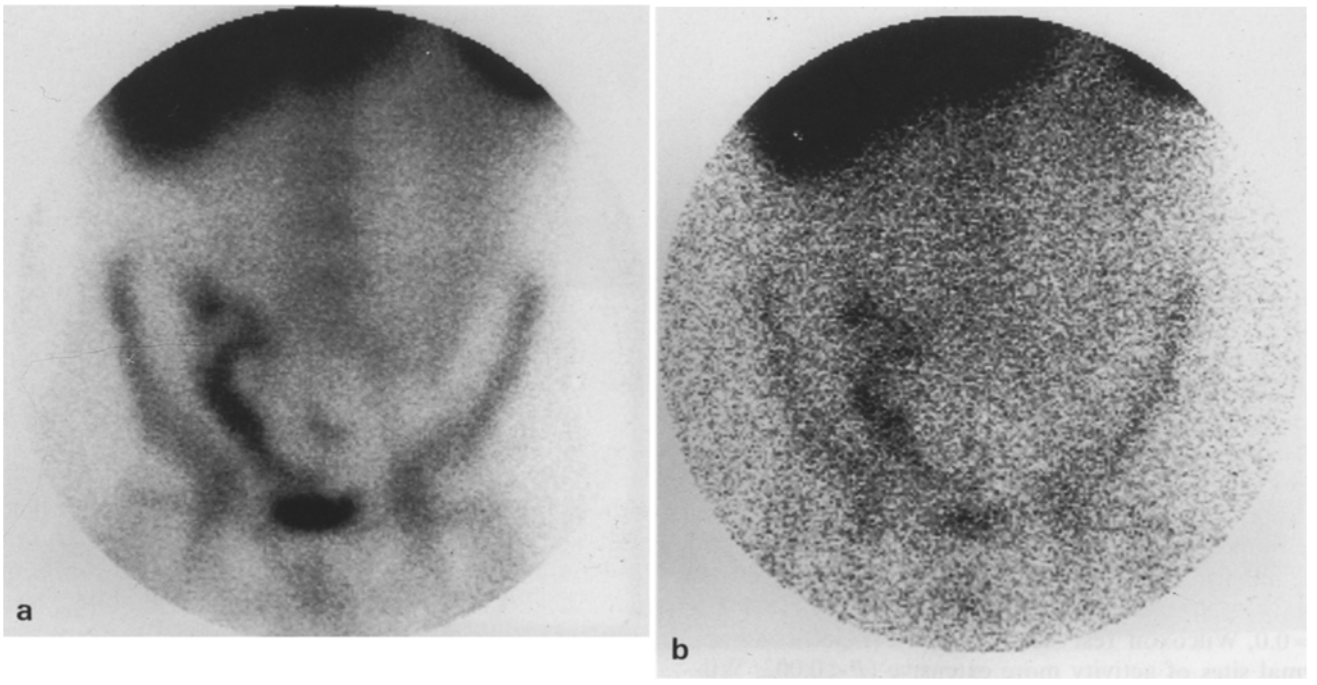

Fig. 1 a, b. One-hour scans of a patient with Crohn's disease showing superior resolution with ^{99m}Tc-HMPAO (**a**) compared with ^{111}In (**b**)

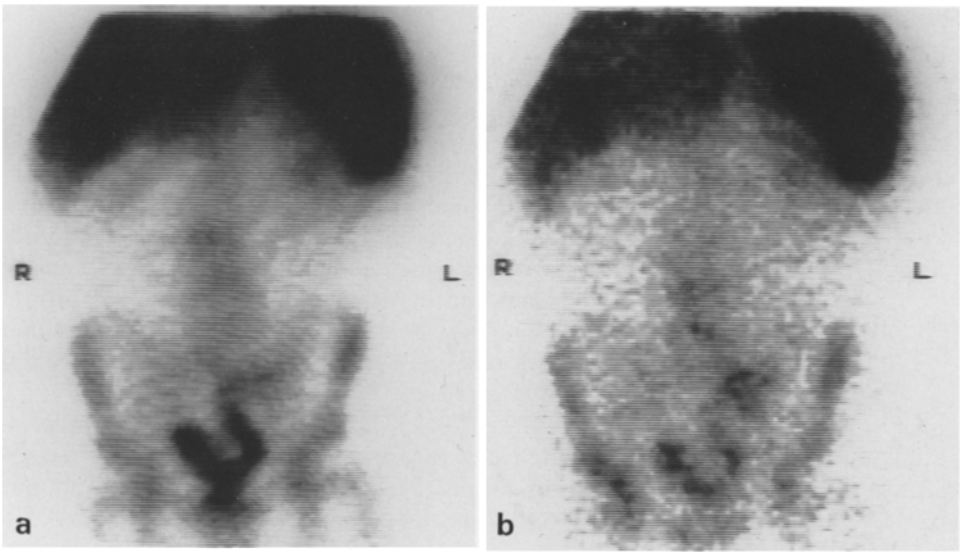

Fig. 2a, b. Three-hour scans of a patient with ileal Crohn's disease showing better resolution and estimation of disease extent with ^{99m}Tc-HMPAO (**a**) compared with ^{111}In (**b**)

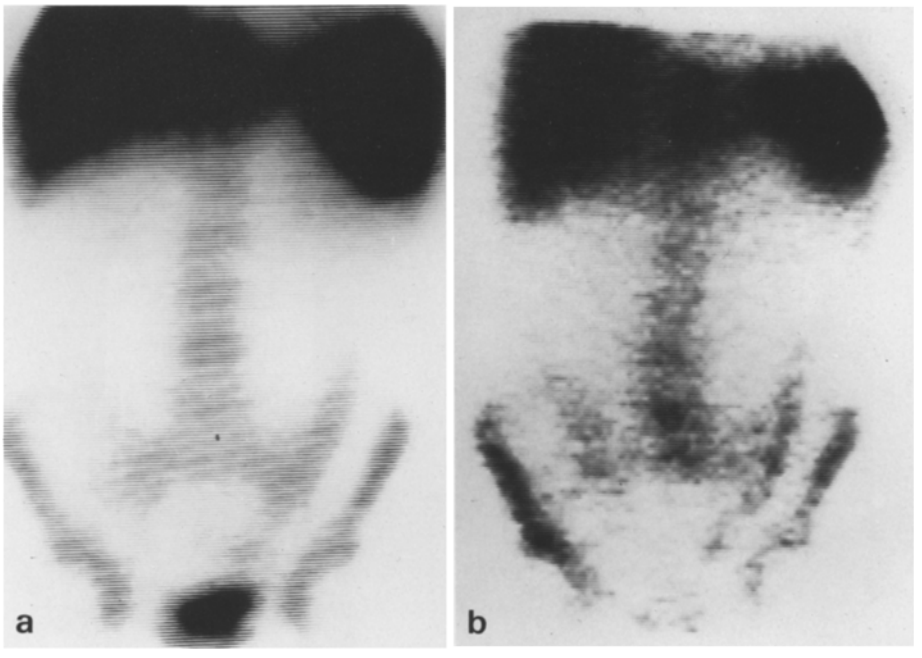

Fig. 3a, b. One-hour ^{99m}Tc-HMPAO (**a**) and 3-h ^{111}In (**b**) scans of a patient with colitis showing similar resolution and assessment of disease extent

WBC scan (Table 1). This patient's ^{99m}Tc-HMPAO WBC scan showed grade 2 activity in the caecal region on the 1-h scan and grade 1–2 activity in the same region at 3 h on the ^{111}In scan. At 1 h abnormal areas were reported as being more extensive with ^{99m}Tc-HMPAO ($P<0.026$, Wilcoxon Test Statistic 24.5) and activity was more intense ($P<0.001$, Wilcoxon Test Statistic 225.0). Thus, both observers were able to localise diseased bowel with more confidence (Fig. 1) using ^{99m}Tc-HMPAO.

Comparing the 3-h scans obtained with both agents, activity was again more intense with ^{99m}Tc-HMPAO ($P=0.0$, Wilcoxon Test Statistic 243.0), (Fig. 2), and abnormal sites of activity more extensive ($P<0.002$, Wilcoxon Test Statistic 5.0).

When the 1-h ^{99m}Tc-HMPAO WBC scans were compared with the 3-h ^{111}In WBC scans there was no significant difference in extent of diseased segments shown ($P<0.384$, Wilcoxon Test Statistic 65.0) or intensity of activity ($P<0.258$, Wilcoxon Test Statistic 205.0) (Fig. 3).

Of the 47 24-h ^{99m}Tc-HMPAO images, 38 (81%) showed diffuse gut uptake; therefore images at this time were not formally compared in all cases.

Physiological bowel activity (i.e. activity not seen on the 1-h ^{99m}Tc-HMPAO WBC scans or the 3-h ^{111}In WBC scans) on the 3-h ^{99m}Tc-HMPAO WBC scans was noticed in 12 out of 47 patients; seven patients had ileal Crohn's disease, two ulcerative colitis and three IBS.

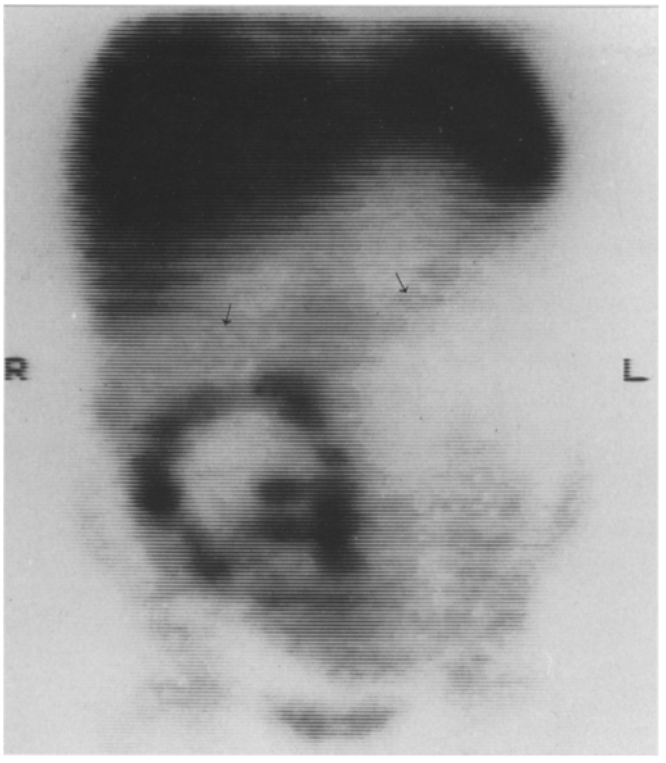

Fig. 4. Three-hour ^{99m}Tc-HMPAO scans of a patient with extensive ileal Crohn's disease and adhesions forming a right iliac fossa mass; physiological bowel activity (*arrowed*) is clearly separate from this process

Apart from two patients with extensive ileal Crohn's disease, this activity was fainter than that associated with diseased bowel (Fig. 4).

Bladder/urinary tract activity at 1 and 3 h did not interfere with interpretation of the ^{99m}Tc-HMPAO WBC images. Cross-talk of ^{99m}Tc into the ^{111}In channel was visualised on the ^{111}In images in two patients: in one of the patients, who had ulcerative colitis, activity was observed in the bladder region, while in the second, prominent pelvicalyceal activity on the ^{99m}Tc-HMPAO WBC image produced faint activity in the regions of the hepatic and splenic flexures on the ^{111}In WBC image. In neither case was the cross-talk into the ^{111}In WBC images sufficiently intense to be misread as active disease.

Discussion

The 2- to 4-h ^{111}In image is currently the "gold standard" for assessing IBD and other agents require to be compared with this. Previous workers [10] have shown a high total agreement rate (71%) for ^{111}In and ^{99m}Tc-HMPAO WBC scans in patients with IBD. The aim of this study was to compare ^{99m}Tc-HMPAO and ^{111}In images directly under the same conditions; agreement was found in 100%. Roddie et al. [4] have reported similar findings, although they did not make a simultaneous comparison. Moreover, we found that the 1-h ^{99m}Tc-HMPAO WBC scans were as accurate as the 3-h ^{111}In WBC scans in detecting active IBD and showed more extensive disease, while a negative scan, in our experience (albeit limited), reliably excluded active disease. The 3-h ^{99m}Tc-HMPAO WBC scans were superior to the 3-h ^{111}In WBC scans, showing more intense activity over involved bowel segments and more extensive activity at disease sites. These differences have been shown despite the fact that the imaging protocol tended to minimise any discrepancies between the two agents. Firstly, the medium energy collimator, because of its thicker septa compared with the low energy collimator (which would have been used in ^{99m}Tc-HMPAO alone had been imaged), caused some reduction in resolution and sensitivity of the ^{99m}Tc-HMPAO WBC images. Secondly, ^{99m}Tc to ^{111}In cross-talk, which was seen in two patients, would have had the effect of enhancing the ^{111}In WBC images. Significant bias towards the ^{99m}Tc-HMPAO images caused by the contribution of ^{111}In activity into the ^{99m}Tc window is ruled out as the ^{99m}Tc-HMPAO images are significantly clearer than the ^{111}In ones at 1 and 3 h; any bias tends towards the ^{111}In image, in view of the more adequate collimation of indium. Nevertheless, the 1-h ^{99m}Tc-HMPAO WBC images were as effective as the 3-h ^{111}In WBC images at assessing IBD. The differences in disease extent may simply be due to more intense activity on ^{99m}Tc-HMPAO WBC scans allowing more segments to be reported with confidence. Others have found good correlation between bowel involvement of ^{99m}Tc-HMPAO WBC scans and barium studies [11], but in the present study barium studies and/or colonoscopy were not performed at the same time as the white cell scans in every patient. Nevertheless, when activity was seen, it occurred at previously demonstrated disease sites. However, in one patient scanned shortly after colonoscopy, the 3-h ^{99m}Tc-HMPAO WBC scan overestimated the extent of the disease compared with both the colonoscopy and ^{111}In. This may have been due to greater sensitivity of ^{99m}Tc-HMPAO in detecting submucosal disease not visible at colonoscopy.

Physiological elimination of ^{99m}Tc-HMPAO via renal and biliary systems and bowel may produce areas of "activity" which reporters should be aware of in order to avoid overinterpreting scans; occasionally extra views such as pelvic outlet views may be necessary. Mountford et al. [7] noted in their study of intra-abdominal sepsis that physiological bowel activity on the 4-h ^{99m}Tc-HMPAO WBC scans significantly reduced the specificity of the study; by 24 h this activity ws indistinguishable from activity due to distal migration of leucocytes from inflamed bowel. However, these authors did not obtain images earlier than 4 h and they studied a different population of patients. In the present study, physiological bowel uptake on the 3-h ^{99m}Tc-HMPAO WBC scans was noted in approximately 25% of patients (12/47) but

it was fainter than and clearly distinguishable from activity associated with areas of diseased bowel and did not interfere with image interpretation (Fig. 4). Nevertheless, we would agree with others [9, 12] that images earlier than 3–4 h are necessary for accurate evaluation of scans, since this activity should not be present on scans performed at 1 h [4].

Early physiological bowel activity is thought to represent biliary excretion of non-cell-bound ^{99m}Tc labelled secondary hydrophilic complexes which become visible as they concentrate in the area of the distal ileum, probably reflecting rapid jejunal and relatively slow ileal transit. Ileal transit might be even slower in the presence of severe ileal disease, leading to accumulation of ^{99m}Tc in the distal ileum. This might explain the slight preponderance of early bowel activity in patients with ileal disease (7/12 patients).

As stated above, it was the aim of this study to directly compare two different white cell labelling techniques under exactly the same conditions, rather than to compare white cell labelling with existing radiological techniques: such a comparison has already been made [2, 6]. It is not possible with the data from this study to make the latter comparison, because most patients did not have their radiological investigations performed at a time sufficiently close to their white cell scans. Comparison of NHS cost estimates shows: ^{111}In scan £ 324, ^{99m}Tc-HMPAO scan £ 410–440, double contrast barium enema £ 61. Comparison of effective dose equivalents (EDE) shows: ^{111}In scan (20 MBq) EDE 12 mSv, uterine absorbed dose 2 mGy, splenic absorbed dose 56 mGy; ^{99m}Tc-HMPAO scan (200 MBq) EDE 3.4 mSv, uterine absorbed dose 0.8 mGy, splenic absorbed dose 31.3 mGy; barium enema EDE 7.69 mSv (range 2.92–33.64 mSv), uterine/ovarian dose 16 mGy [13].

We conclude that, in terms of isotope availability, radiation dosimetry, image quality and diagnosis possible as early as 1 h, ^{99m}Tc-HMPAO is the agent of choice in assessing the extent and activity of inflammatory bowel disease. However, early imaging is required for optimal interpretation of scans.

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